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Occurrence of a Nucleolar-Like Body in Lotus Corniculatus L.

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Occurrence of a Nucleolar-Like Body in *Lotus Corniculatus* L.[1]

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Abstract: A genotype of the variety Empire *Lotus corniculatus* L. was found containing an organelle which appeared as an extra nucleolus within the nucleus. In microspore mother cells this organelle first appeared at early prophase I, staining much more intensely than the normal nucleolus and remained until mid-diakinesis, at which time it disappeared. This cycle coincided with that of the normal nucleolus. It has not been determined if the nucleolar-like body arises from a nucleolar organizer as does the normal nucleolus. This organelle was not associated with any chromosome in 16 per cent of the microspore mother cells examined at stages from pachynema to diakinesis. This nucleolar-like body was transmitted through the pollen of a plant containing the organelle. The pattern of inheritance of this organelle is presently under study.

In most living organisms definite regions exist on one or more chromosomes of the basic chromosome complement which are responsible for the organization of the nucleolus of the cell. This region, called a nucleolar organizer, is usually confined to only one chromosome in most species, but occasionally the activity is found on additional chromosomes.

In somatic tissue it is not uncommon to find a nucleolus for each chromosome carrying a nucleolar organizer. In dividing endosperm tissue of maize (3N), Duncan and Ross (1) found three nucleoli at telophase. However, by interphase they have usually fused into one. Two nucleoli of dissimilar size were often present, but more than three were not observed.

In barley microspore mother cells nucleolar organizing regions were found present in two chromosomes of the genome (VI & VII) and approximately six per cent of the sporocytes examined had two nucleoli (2). However, in *Spinacia oleracea* L. Bemis and Wilson (3) found that two pairs of chromosomes were associated with the nucleolus at diakinesis. This indicated that two different chromosomes in the genome had nucleolar organizers although only one nucleolus was formed. Navashin (4) observed a competitive activity of the nucleolar organizers of different species of *Crepis*. In certain species crosses this competition was so great that the secondary constriction of one species failed to develop. However, the capacity of development was regained under conditions of reduced nucleolar organizer competition.

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In maize it has been shown that the structural entity of the nucleolar organizing region is not required to retain its organizational capacity. McClintock (5) has shown that the nucleolar organizer can be broken into smaller portions each capable of forming a nucleolus. A 6-9 translocations was obtained in which the break in chromosome 6 occurred in the nucleolar organizer. Somatic tissue of plants homozygous for the interchange frequently possessed four nucleoli. In the absence of any nucleolar organizer the nucleolus material formed small droplets.

Lin (6) synthesized plants with variable numbers of nucleolar organizers by selfing a stock heterozygous for a reciprocal translocation of chromsome 6 and a "B" chromosome. The break in chromosome 6 occurred through the nucleolar organizer. At pachynema in plants with six B^6 chromosomes, all were found to be attached to a single nucleolus.

In a study of meiosis in *Lotus corniculatus* L. a genotype of the Empire variety was found whose microspore mother cells possessed a dark-staining, nucleolar-like body in addition to the normal nucleolus. The staining reaction, size, and relation of this nucleolar-like body to the chromosomes is the subject of this report.

Materials and Methods

Umbels of plants to be examined cytologically were collected when the florets were 0.14 – 0.16 mm in length. They were placed in a Carnoy solution (6 parts ethanol: 3 parts chloroform: 1 part glacial acetic acid) for a two-hour period. The umbels were then stored in 70 per cent ethanol until examined. Anthers were dissected from the florets, and microspore mother cells were stained with aceto-carmine, propiono-carmine, and the Feulgen technique.

Results and Discussion

The nucleolar-like body appears at early prophase I of meiosis at approximately the same time that the normal nucleolus becomes highly stainable. Upon staining with aceto-carmine or propiono-carmine, the nucleolar-like body in miscrospore mother cells stains much more intensely and is easily distinguishable from the normal nucleolus (see figure 1).

The organelle appears to grow slightly until a maximum size is attained at diplonema. At this time the nucleolar-like body is usually the same size as the normal nucleolus. However, in some microspore mother cells it is smaller than the normal nucleolus. The nucleolar-like body diminishes in size and staining intensity at diakinesis and disappears completely by the middle of this stage. The time of disappearance closely parallels that of the normal nucleolus, and at this time the two are often indistin-

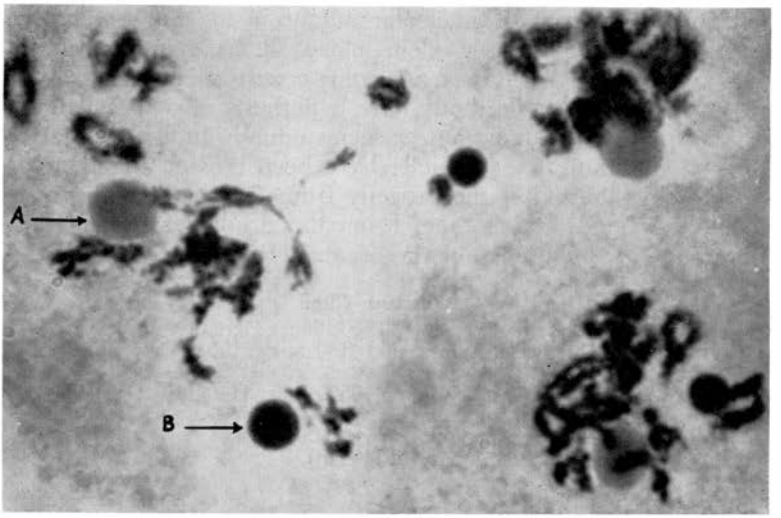

Figure 1. Early diakinesis in a microspore mother cell of *Lotus corniculatus* L. Normal nucleolus (A) and the nucleolar-like body (B).

guishable. In telophase of the first meiotic division the nucleolus of *Lotus* does not reappear. However, the interval between divisions is so short that it would escape observation. This is unlike other species. The normal nucleolus reappears in the interphase nucleus of the microspore and occasionally two nucleoli are present. This phenomenon is not considerd unusual, since this species is a tetraploid and the microspore nucleus does contain two nucleolar organizers. The nucleolar-like body was not observed in the interphase nucleus of the microspore. An extensive study, however, has not been made.

In the only cross of the original clone containing the nucleolar-like body as the pollen parent to a normal plant, two F_1 plants were obtained. In a cytological examination of these plants, the nucleolar-like body was found.

These observations indicate that the organelle may be inherited as a genetic dominant or may arise at a specific organizer site as does the normal nucleolus. Presently, staining techniques are not adequate to provide suitable pachynema preparations for critical examination of chromosome nucleolus associations. In 107 of a total of 669 cells, or 16 per cent of those observed, the nucleolar-like body was not closely associated with any chromosome. This may have been due to the pressure applied to the slide in the smearing process, since the normal nucleolus was occasionally found to be free of its nucleolar organizer.

Fusion of the normal nucleolus with the nucleolar-like body was never observed in the material. Thus the possibility that this new organelle could have been due to a translocation of a

portion of the normal nucleolar organizer to another chromosome, as has been observed in maize (5, 6), seems remote at this time. Since the normal nucleolus occurred in all cells along with the nucleolar-like body, any hypothesis of a deficiency of the normal nucleolar organizer seems equally unlikely. The two plants containing the organelle have been crossed together and to normal plants, but the progeny from these crosses have not been examined at this time. From the results of these crosses the mode of inheritance of the organelle will be determined.

Literature Cited

1. Duncan, R. E., and J. G. Ross, 1950. Jour. Heredity 41:259-268.
2. Burnham, C. R., F. H. White, and R. Livers. 1954. Cytologia 19:191-202.
3. Bemis, W. P. and G. B. Wilson. 1953. Jour. Heredity 44:91-95.
4. Navashin, M. 1934. Cytologia 5:169-203.
5. McClintock, B. 1934. Z. Zellforsch. 21:294-328.
6. Lin, Mei. 1955. Chromosoma 7:340-370.

The Demonstration of Mitotic Figures in Green Algae

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Abstract: Wittman's aceto-iron-haematoxylin stain was combined with Hoyer's mounting medium in a rapid method for demonstrating mitosis in the green algae. Both filamentous and unicellular forms were killed, fixed, and stained in one step with the self-mordanting stain. Cells were then washed with acetic acid and transferred directly to the water-soluble Hoyer's medium. Slides prepared in this manner are permanent. Chlorophyll was bleached from the algal cells by this medium. This revealed the stained nuclei and mitotic figures. This simplified technique is particularly suitable for student use.

The study of mitosis in the green algae is relatively difficult as compared to similar studies with the higher plants. Numerous cytological methods, e.g., aceto-carmine (1) and propionocarmine (2), have been employed successfully for chromosome counts but they are not very practical for student use. They involve the preliminary steps of killing and fixing the cells, followed by the bleaching of chlorophyll from the cells before staining. Plasmolysis and collapse of cells is frequently encountered when it is attempted to make these preparations permanent. A simple and practical cytological technique which eliminates these problems is described.

PROCEDURE

The combination of Wittman's aceto-iron-haematoxylin stain

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